Part V. Enzymes of Polydesoxyribonucleotide Metabolism

ENZYMATIC SYNTHESIS OF DESOXYRIBONUCLEIC ACID*

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Our studies during the past 3 years on the synthesis of desoxyribonucleic acid (DNA) by an enzyme that we have partially purified from *Escherichia coli* have revealed the following basic features of this reaction.

(1) The desoxynucleoside triphosphates† of the 4 predominant bases found in DNA—adenine, guanine, thymine, and cytosine—must be present for appreciable synthesis to occur.

(2) Polymerized DNA and Mg^{++} are essential for the reaction.

(3) Inorganic pyrophosphate is released in amounts equal to the amount of nucleotide incorporated into DNA.[1-3]

On the basis of these findings, the over-all equation for the synthesis of DNA by this enzyme may be formulated in the following way:

$$n\begin{bmatrix}\text{dTPPP}\\ \text{dGPPP}\\ \text{dAPPP}\\ \text{dCPPP}\end{bmatrix} + \text{DNA} \xrightleftharpoons{Mg^{++}} \text{DNA} - \begin{bmatrix}\text{dTP}\\ \text{dGP}\\ \text{dAP}\\ \text{dCP}\end{bmatrix}_n + 4(n)\text{PP}$$

In this report I shall describe in some detail experiments in which we have attempted a characterization of the enzymatically synthesized DNA with regard to its physical and chemical properties. In addition, evidence will be presented for the enzymatic synthesis, under rather specialized conditions, of a DNA-like copolymer of desoxyadenylate and thymidylate.

This enzyme, which in the presence of a DNA primer catalyzes the polymerization of the 4 desoxynucleoside triphosphates, has been purified about two to four thousandfold relative to the crude extract of *E. coli* that serves as its source material. With such preparations net synthesis of DNA can be demonstrated readily by a variety of procedures (TABLE 1). In the first experiment shown in the table, an increase in DNA of somewhat more than twofold was observed as measured by isotope incorporation, ultraviolet spectrophotometry, or desoxypentose estimation. In the other 3 experiments there were increases in DNA of from ten- to twentyfold, so that 90 to 95 per cent of the isolated DNA had its origin in the desoxynucleoside triphosphates supplied in the reaction. Since the enzymatically synthesized DNA shows the very high viscosity characteristic of DNA isolated from natural sources,[3, 4] one can measure the net synthesis of DNA in yet another way—that is, by viscometry. Thus, one simply incubates the various components of the reaction in a conventional Ostwald viscometer placed in a thermostated bath, and follows the

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† The abbreviations used in this report are as follows: dATP or dAPPP, desoxyadenosine triphosphate; dCTP or dCPPP, desoxycytidine triphosphate; dGTP or dGPPP, desoxyguanosine triphosphate; and dTTP or dTPPP, thymidine triphosphate.

increase in viscosity as a function of time. As shown in FIGURE 1, the viscosity of the reaction mixture continued to increase for approximately 3 hours, then stopped abruptly, probably as a result of the exhaustion of the substrates; it then underwent a gradual decline. This slow decrease in viscosity is most probably attributable to the presence of a small amount of desoxyribonuclease activity that is present even in our most highly purified enzyme preparations.

The availability of enzymatically synthesized DNA having 90 to 95 per cent of its origin in the desoxynucleotide substrates has permitted a study of its

TABLE 1
NET SYNTHESIS OF DNA

Experiment*	Estimation	Control (no enzyme) μmoles	Complete μmoles	Δ μmoles
1	P^{32} incorporation	0.00	0.28	0.28
	Optical density	0.19	0.46	0.27
	Desoxypentose	0.19	0.40	0.21
2	Optical density	0.06	0.63	0.57
3	Optical density	0.05	0.58	0.53
4	Optical density	0.05	0.64	0.59
5	Optical density	0.04	0.87	0.85

* In Experiment 1, the incubation mixture (3.0 ml.) contained 0.15 μmole of $dAP^{32}PP$ (1.3×10^6 cpm/μmole), 0.3 μmole of dGTP, 0.15 μmole of dCTP, 0.15 μmole of dTTP, 200 μmoles of potassium phosphate buffer (*p*H 7.4), 20 μmoles of $MgCl_2$, 0.1 mg. of calf thymus DNA, and 12 μg. of Enzyme Fraction VII.[2] The mixture was incubated at 37° C. for 180 min. DNA was precipitated, washed, taken up in 1.2 ml. of 0.5 *N* perchloric acid, and heated for 15 min. in a boiling-water bath. Optical density measurements were made at 260 mμ and converted to nucleotide equivalents, using a molar extinction coefficient of 8960 (derived from the calculated values for an acid hydrolyzate of calf thymus DNA). In the P^{32} estimation of DNA synthesis, incorporation of desoxyadenylate was multiplied by a factor based on its percentage composition in calf thymus DNA. The radioactivity actually observed for the controls did not exceed the background count. In Experiments 2, 3, 4, and 5, the reaction mixture (1.0 ml.) contained 0.32 μmole of each of the 4 triphosphates, 30 μg. of calf thymus DNA, 60 μmoles of potassium phosphate buffer (*p*H 7.4), 6 μmoles of $MgCl_2$, and 8 μg. of Enzyme Fraction VII. The mixture was incubated for 250 min at 37° C., and 2 M NaCl was then added to give a final concentration of 0.2 M; the mixture was heated for 5 min. at 70° C. Unreacted triphosphates were removed by exhaustive dialysis against 0.2 M NaCl. The product contained no acid-soluble material. Optical density at 260 mμ was determined and converted to nucleotide equivalents using a molar extinction for DNA of 6900.

physical dimensions.[4] In general, such studies have shown that the enzymatically produced material has essentially the same properties as DNA carefully isolated from natural sources. These results are summarized in TABLE 2. The average sedimentation coefficients observed for a large number of runs ranged from 20 to 25 Svedberg units. Measurements of reduced viscosity have yielded values of 15 to 45 dl./gm., which are comparable to those observed for calf thymus DNA (40 to 50 dl./gm.). On the basis of these parameters, we have calculated average molecular weights of from 4 to 6 million for a number of synthetic products. The viscosity and sedimentation behavior of enzymatically synthesized DNA would suggest that it is organized as relatively stiff macromolecules, with effective volumes that are greater than would be

expected from single polynucleotide chains with freedom of rotation at each link in the backbone. Supporting this view are the observations made when the DNA product was heated at 100° C. for 15 min. Under these conditions, the rigid structure of DNA is known to collapse.[5] Like calf thymus DNA, the viscosity of the enzymatic product decreased to less than 1 (grams per 100 ml.)$^{-1}$ and the sedimentation rate decreased only slightly to 14 S. Further-

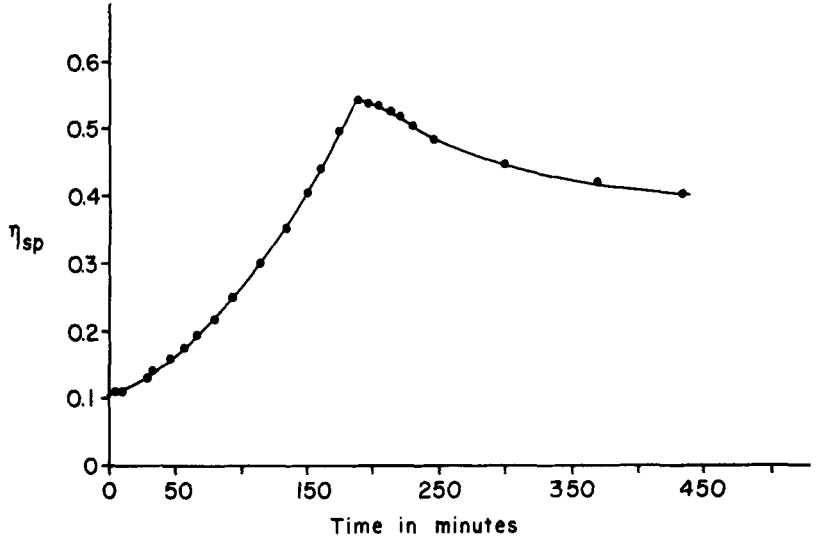

FIGURE 1. Measurement of DNA synthesis by viscometry. The incubation mixture was the same as that used in experiments 2 to 5, TABLE 1. Incubation was at 37° C.

TABLE 2
PHYSICAL PROPERTIES OF ENZYMATICALLY SYNTHESIZED DNA

	Primer	Product	Heated at 100° C., 15 min.	
			Primer	Product
Sedimentation coefficient	25	20–25	20	14
Intrinsic viscosity, dl./gm.	40–50	15–45	<1	<1
Molecular weight	8×10^6	$4–6 \times 10^6$		

more, before heating, the product showed the typical hypochromicity characteristic of a highly ordered DNA molecule.[6] Upon digestion with crystalline pancreatic desoxyribonuclease, an increase in ultraviolet absorption was produced at the same rate and to the same extent (30 per cent above the starting value) as that observed for calf thymus DNA (FIGURE 2).

With respect to the chemical composition of the enzymatically synthesized DNA, it has already been demonstrated that this DNA consists of desoxynucleotides linked by typical 3′-5′ phosphodiester bonds, and all 4 of the desoxynucleotides that occur naturally in DNA are present.[3] The Watson-

Crick[7, 8] model for DNA, however, would predict that certain base substitutions could be made provided the proper hydrogen-bonding relationships are maintained. Thus, one would expect that thymine could be replaced by uracil or bromouracil, since both of these analogues maintain the keto grouping in the 6 position and the H in the 1 position necessary for hydrogen bonding with the corresponding 6 amino and 1-N groups of adenine (FIGURE 3). Similarly, methylcytosine or bromocytosine might substitute for cytosine, and hypoxanthine may replace guanine. In the experiment summarized in TABLE 3

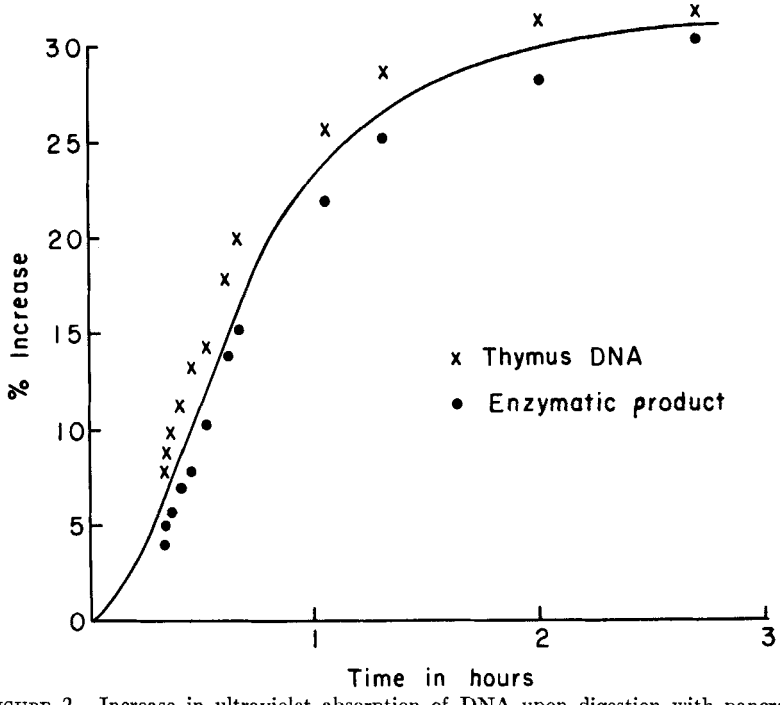

FIGURE 2. Increase in ultraviolet absorption of DNA upon digestion with pancreatic desoxyribonuclease.

the desoxynucleoside triphosphates of the various purine and pyrimidine analogues were prepared chemically and tested for their ability to replace dATP, dCTP, dGTP, and dTTP as substrates in the enzymatic synthesis of DNA. As indicated, the triphosphates of 5-bromodesoxyuridine and desoxyuridine replaced only dTTP. Similarly, 5-methyldesoxycytidylate and 5-bromodesoxycytidylate were incorporated specifically in place of dCTP. Desoxyinosinate replaced only desoxyguanylate, although at an appreciably reduced rate. These results, then, are clearly in accord with the specific base-pairing requirements of the Watson-Crick theory.

Two further questions can be posed with regard to the chemical composition of the enzymatically synthesized DNA: (1) Does this DNA show the equivalence of purines to pyrimidines observed in all naturally occurring DNAs?[9]

(2) Does the base composition of the DNA primer influence the composition of the synthesized product? To answer these questions a number of products were prepared, differing only in the source of DNA used as primer in the synthesis. The base compositions of the various primers and products were determined by the method of Wyatt and Cohen;[10] the results of these analyses

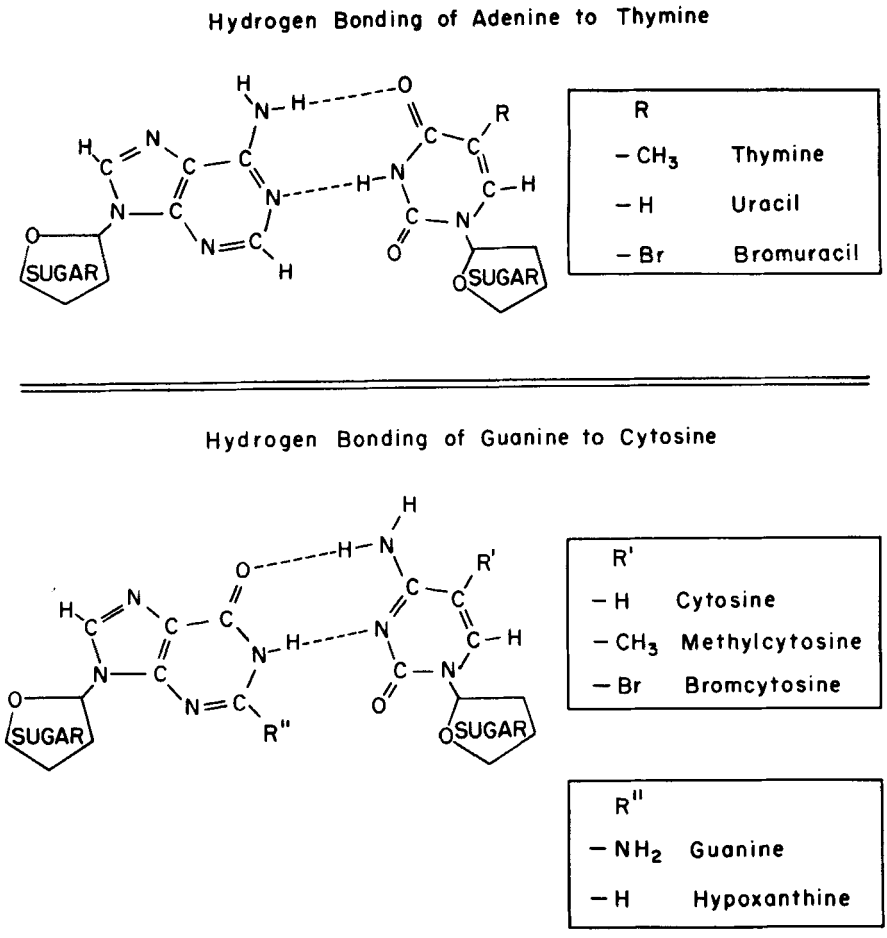

FIGURE 3. Hydrogen bonding of "natural" and "unnatural" purine and pyrimidine bases.

are summarized in TABLE 4. In each enzymatically synthesized DNA sample a close correspondence was observed between the content of adenine and thymine on the one hand, and guanine and cytosine on the other, so that the total amount of purine was in each case the same as the total amount of pyrimidine (that is, A = T; G = C; A + G = T + C). Furthermore, the ratio A+T/G+C in the enzymatic product was always close to that of the primer used in its synthesis. The primer values ranged from 0.49 for *M. phlei* to greater than 40 for the enzymatically synthesized copolymer of desoxyadenylate

and thymidylate. It is noteworthy that the net increase in DNA was in each case at least 10 times the primer added except in the *M. phlei*, where it was only fourfold. Thus, in all but the *M. phlei* samples, more than 90 per cent of the bases was derived from the nucleotide substrates for the reaction. It may be noted in TABLE 4 that the ratio of A+T/G+C was somewhat higher than that for the primer in some products; lower ratios have never been observed. It seems most probable that these divergencies between primer and product values are the result of contamination of the products with the desoxyadenylate-thymidylate (A-T) copolymer, which is produced after lag periods of from

TABLE 3

REPLACEMENT OF NATURAL BASES BY ANALOGUES IN ENZYMATIC SYNTHESIS OF DNA

Experiment	Control value* (mμmoles)	Base analogue used	Natural base omitted			
			Thymine	Adenine	Guanine	Cytosine
			(percentages of control)†			
1	0.50	Uracil	54	4	6	
1a	0.88	Uracil				3
2	0.43	5-Bromouracil	97	2	4	
2a	0.42	5-Bromouracil				4
3	0.51	5-Bromocytosine		4	4	118
3a	0.40	5-Bromocytosine	4			
4	0.58	5-Methylcytosine		2	3	185
4a	0.52	5-Methylcytosine	2			
5	0.37	Hypoxanthine		3	25	5
5a	0.27	Hypoxanthine	4			

* Control values are millimicromoles of radioactive desoxynucleotide incorporated into DNA in the absence of analogue. Incubation mixtures contained, in 0.3 ml., 5 mμmoles each of dTTP, dATP, dCTP, and dGTP; 2 μmoles of $MgCl_2$; 20 μmoles of potassium phosphate (*p*H 7.4); 10 μg. of calf thymus DNA; and 1 μg. of Enzyme Fraction VII.[2] Labeled substrates were: $dCP^{32}PP$ in Experiments 1, 2, and 5a; $TP^{32}PP$ in Experiments 1a, 3, 4, and 5; and $dGP^{32}PP$ in Experiments 2a, 3a, 4a. After incubation for 30 min. at 37° C., incorporation of isotope into DNA was measured as reported for assay of polymerase.[2]

† The percentage value represents the fraction of the labeled substrate incorporated when the analogue (5 mμmoles) was used instead of a base. All bases, natural or analogue, were supplied as the desoxynucleoside triphosphate. Values of 5 per cent or less are near the limit of detectability and are of questionable significance.

3 to 6 hours. The synthesis and properties of this polymer will be discussed in more detail below.

The results shown in TABLE 4 were obtained with equimolar concentrations of the 4 desoxynucleoside triphosphates in the synthetic reaction. Similar results were observed with markedly distorted substrate concentrations. In the experiment summarized in TABLE 5, the initial concentration of dTTP or of both dTTP and dATP was lowered to one fifth the level of the other triphosphates. As the reaction proceeded, the disparity in relative concentrations was exaggerated and, presumably, the extent of DNA synthesis was curtailed by the exhaustion of the limiting substrate. Nevertheless, the product synthesized maintained the same A+T/G+C ratio as the primer, and the purine content was equivalent to the pyrimidine content. Finally, the base ratios of

the synthesized product were found to be essentially the same regardless of whether it was isolated early in the reaction or after synthesis had stopped. To determine whether this result held true even at the earliest stages of reaction,

TABLE 4
PURINE AND PYRIMIDINE COMPOSITION OF ENZYMATICALLY SYNTHESIZED DNA*

DNA	No. of analyses	A	T	G	C	$\frac{A+T}{G+C}$	$\frac{A+G}{T+C}$
M. phlei							
Primer	3	0.65	0.66	1.35	1.34	0.49 (0.48–0.49)	1.01 (0.98–1.04)
Product	3	0.66	0.80	1.17	1.34	0.59 (0.57–0.63)	0.85 (0.78–0.88)
A. aerogenes							
Primer	1	0.90	0.90	1.10	1.10	0.82	1.00
Product	3	1.02	1.00	0.97	1.01	1.03 (0.96–1.13)	0.99 (0.95–1.01)
E. coli							
Primer	2	1.00	0.97	0.98	1.05	0.97 (0.96–0.99)	0.98 (0.97–0.99)
Product	2	1.04	1.00	0.97	0.98	1.02 (0.96–1.07)	1.01 (0.96–1.06)
Calf thymus							
Primer	2	1.14	1.05	0.90	0.85	1.25 (1.24–1.26)	1.05 (1.03–1.08)
Product	6	1.19	1.19	0.81	0.83	1.46 (1.22–1.67)	0.99 (0.82–1.04)
T2 bacteriophage							
Primer	2	1.31	1.32	0.67	0.70	1.92 (1.86–1.97)	0.98 (0.95–1.01)
Product	2	1.33	1.29	0.69	0.70	1.90 (1.82–1.98)	1.02 (1.01–1.03)
Synthetic A-T copolymer	1	1.99	1.93	<0.05	<0.05	>40	1.05

* The reaction mixtures and the procedure for isolation of the products were the same as those described for Experiments 2 to 5, TABLE 1. A, T, G, and C refer to adenine, thymine, guanine and cytosine, respectively, except that C in the case of T_2 bacteriophage primer refers to hydroxymethylcytosine. The values given represent averages of the number of analyses indicated. The figures in parentheses are the ranges of values obtained.

TABLE 5
EFFECT OF RELATIVE SUBSTRATE CONCENTRATIONS ON COMPOSITION OF SYNTHETIC DNA

Substrates (relative molar concentration)				Net synthesis	Products	
dCTP	dGTP	dTTP	dATP		$\frac{A+G}{C+T}$	$\frac{A+T}{G+C}$
1.0	1.0	1.0	1.0	11×	1.00	1.98
1.0	1.0	0.2	1.0	6×	1.04	1.82
1.0	1.0	0.2	0.2	6×	0.97	1.82
T_2 bacteriophage DNA as primer					0.98	1.92

isotopically marked substrates were used to distinguish the newly synthesized DNA. In such an experiment, 2 identical reaction mixtures were set up, 1 containing C^{14}-dCTP and the other C^{14}-dTTP. The amount of enzyme and the length of incubation were adjusted to permit an increase of DNA of as little as 2 per cent relative to the DNA added as primer. After incubation, aliquots were removed and assayed for incorporation of isotope into DNA. As shown in TABLE 6, the T/C ratio of the product was similar to that of the primer when

net increases were small (2 to 63 per cent), or large (>1000 per cent, as in TABLE 4).

From these findings, as well as from the observations on the specific replacement of the naturally occurring desoxynucleotide substrates by related analogues, it is clear that the equivalence of adenine to thymine and guanine to cytosine is an inherent feature of DNA synthesis by the "polymerase" enzyme of *E. coli*. Furthermore, the data suggest that the DNA added to the reaction is serving as a template for the enzymatic replication of DNA.

TABLE 6

BASE RATIOS OF THE PRODUCT ISOLATED EARLY IN THE REACTION AS DETERMINED WITH ISOTOPICALLY MARKED SUBSTRATES*

Primer DNA	Increase in DNA, per cent	T incorporated, mμmoles	C incorporated, mμmoles	T/C ratios	
				Product	Primer
M. phlei	2	0.047	0.11	0.42	0.49
M. phlei	35	0.97	2.05	0.47	
Calf thymus	8	0.40	0.28	1.43	1.24
Calf thymus	63	3.08	2.43	1.27	
A. aerogenes	18	0.74	0.93	0.79	0.82

* Duplicate reaction mixtures, as described in TABLE 1 for experiments 2 to 5, were used in each experiment with the exception that C^{14}-dTTP was used in 1 vessel and C^{14}-dCTP was used in the second. After the appropriate incubation period, aliquots were removed and incorporation of isotope into DNA was determined as reported for assay of polymerase.[2]

TABLE 7

ENZYMATIC SYNTHESIS OF VISCOUS DNA FROM NONVISCOUS PRIMERS

	Thymus DNA	Heated thymus DNA	ΦX174 DNA
Reaction:			
Rate	1×	2×	2×
Extent	~ tenfold	~ tenfold	~ tenfold
Viscosity: (dl./gm.)			
Primer	45		1
Product	41	20	22
Heated product	<1	—	<1
% Hyperchromic effect:			
Primer	30	—	10
Product	30	—	30

In view of the possible participation of the DNA primer as a template in the synthetic reaction, it is pertinent to ask to what extent the size and structure of the DNA primer influence the enzymatically synthesized product. It has already been reported that extensive degradation of DNA by the action of pancreatic desoxyribonuclease or dilute acid destroys its priming capacity.[3] [11] On the other hand, heat treatment (100° C. for 10 min.) of calf thymus DNA, resulting in collapse of its macromolecular structure as evidenced by a 30 per cent hyperchromicity and loss of viscosity, produces a primer that supports DNA synthesis at twice the rate and to the same extent as the unheated material (TABLE 7); moreover, a viscous product results. Essentially analogous results were observed using the low molecular weight and comparatively

nonviscous DNA isolated by Sinsheimer from the small phage ΦX174.[12] Again, the synthetic rate was approximately twice that observed with calf thymus DNA and the extent of synthesis was 10 times that of the added primer. Starting with a primer with reduced viscosity of the order of 1 dl./gm., a product was synthesized with a viscosity more than twentyfold that of its primer. Such a product responded to heating or the action of pancreatic desoxyribonuclease in a manner quite comparable to native calf thymus DNA. Thus, upon heating, the viscosity of the product dropped from a value of 22 to less than 1 (grams per 100 ml.)$^{-1}$ and, upon treatment with pancreatic desoxyribonuclease, a 30 per cent hyperchromic effect was observed, as opposed to the 10 per cent hyperchromicity characteristic of the ΦX174 DNA primer.

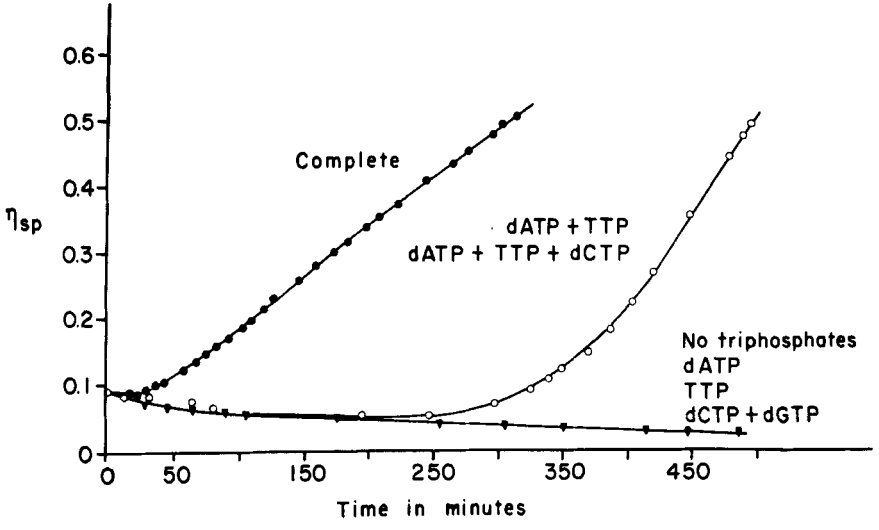

FIGURE 4. Synthesis of DNA and of desoxyadenylate-thymidylate copolymer, with thymus DNA as primer.

It is clear from these results that a collapsed DNA molecule is a highly efficient primer for DNA synthesis by the *E. coli* polymerase and will initiate synthesis of the organized, hydrogen-bonded structure characteristic of most native DNAs. Of further interest in this regard is the observation already noted[3] that treatment of a primer with minute amounts of crystalline pancreatic desoxyribonuclease

$$\left(\frac{\mu g.\ DNA}{\mu g.\ desoxyribonuclease} = 2 \times 10^5\right)$$

results in a two- to threefold stimulation in synthetic rate. The decision, however, as to whether disruption, or at least some modification, of the macromolecular organization of the primer is essential for DNA synthesis in this system must await an enzyme preparation that is completely free of desoxyribonuclease activity.

To return to a consideration of the desoxyadenylate-thymidylate (A-T) copolymer briefly referred to earlier in the paper: as shown in FIGURE 4, a

viscous polymer was formed in the presence of dATP and dTTP after a lag period of about 4 hours. A similar polymer is formed even in the absence of added DNA. Measurements of polymer synthesis during this interval (by conversion of radioactive dATP or dTTP into an acid-insoluble form) demonstrated a comparable lag. We have not observed formation of the corresponding desoxyguanylate-desoxycytidylate (G-C) copolymer. Once formed and isolated, the A-T polymer can initiate the synthesis of a new polymer with no time lag (FIGURE 5). As shown in TABLE 4, the A-T polymer consists exclusively of desoxyadenylate and thymidylate despite the presence during synthesis of all 4 of the desoxynucleoside triphosphates. Physical examination of such polymers has shown that they have the sedimentation properties and viscosity characteristic of native DNA.

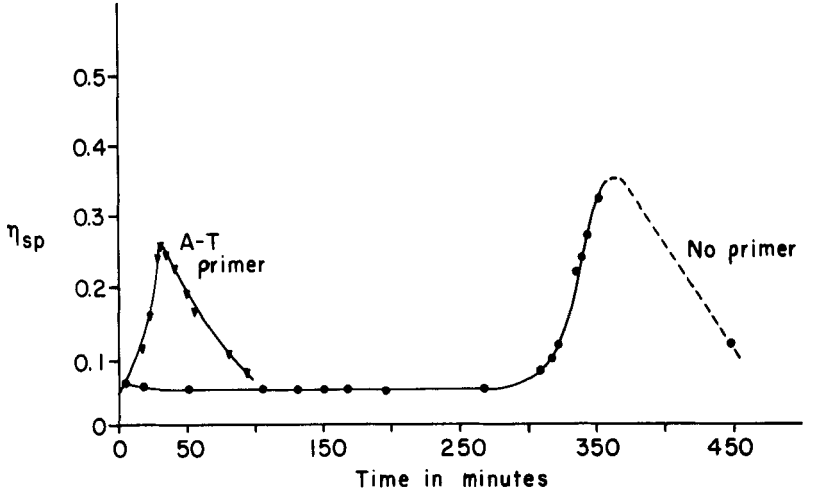

FIGURE 5. Synthesis of desoxyadenylate-thymidylate copolymer in the presence and absence of an A-T primer.

A unique feature of the A-T polymer is its extreme responsiveness to desoxyribonuclease activity. Thus, once synthesis of the polymer has ceased, it is very rapidly degraded to a nonviscous (and acid-soluble) form by the nuclease activity present in the polymerase preparation (FIGURE 5). It is conceivable that our failure to observe synthesis of a G-C polymer is merely a manifestation of the even greater responsiveness of such a polymer to nuclease action.

Perhaps one of the most interesting and puzzling features of this reaction is the significance of the prolonged lag period that precedes initiation of synthesis. One might speculate that it is during this interval that the appropriate primer is somehow developed. Although no acid-insoluble materials can be detected during this period, a search for small polynucleotides might well prove fruitful.

Summary

Physical characterization of the DNA synthesized by the *E. coli* polymerase in the presence of 4 desoxynucleoside triphosphates and a DNA primer has

shown it to have the basic structural features commonly associated with DNA carefully isolated from natural sources. It has a sedimentation coefficient of approximately 25 S and a reduced viscosity ranging from 15 to 45 dl./gm. A 30 per cent hyperchromic effect results upon treatment with pancreatic desoxyribonuclease.

Chemical analysis of the enzymatically synthesized DNA shows it to possess the equivalence of adenine to thymine and guanine to cytosine ratios required by the Watson-Crick model for DNA. In confirmation of this result is the finding that the desoxynucleoside triphosphates of the purines and pyrimidines that occur naturally in DNA can be replaced specifically in the enzymatic reaction only by those analogues whose hydrogen-bonding capacities are in accord with the Watson-Crick model. The ratio of A+T/G+C in the enzymatic product is very close to that of the primer DNA used in its synthesis; primer values have ranged from 0.50 to greater than 40. The values obtained are independent of the relative concentrations of the desoxynucleotide substrates used in the synthesis, or of the extent of net synthesis. These results have led to the hypothesis that the DNA primer participates in the synthetic reaction directly as a template.

DNA that has undergone collapse of its secondary structure as a result of heating, as well as the DNA of relatively small molecular weight from the small phage ΦX174 will support efficient synthesis of a viscous, hypochromic product.

The *E. coli* polymerase is capable, after a time lag of 3 to 6 hours, of synthesizing, in the absence of added DNA, a viscous DNA-like copolymer of desoxyadenylate and thymidylate. Such a polymer, in turn, can initiate, with no lag, synthesis of a new polymer and, despite the presence of all 4 desoxynucleotide substrates in the reaction, will contain exclusively desoxyadenylate and thymidylate.

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References

1. KORNBERG, A. 1957. Pathways of enzymatic synthesis of nucleotides and polynucleotides. *In* The Chemical Basis of Heredity. : 579–614. W. D. McElroy and B. Glass, Eds. Johns Hopkins Press. Baltimore, Md.
2. LEHMAN, I. R., M. J. BESSMAN, E. S. SIMMS & A. KORNBERG. 1958. Enzymatic synthesis of deoxyribonucleic acid. I. Preparation of substrates and partial purification of an enzyme from *E. coli*. J. Biol. Chem. **233:** 163–170.
3. BESSMAN, M. J., I. R. LEHMAN, E. S. SIMMS & A. KORNBERG. 1958. Enzymatic synthesis of deoxyribonucleic acid. II. General properties of the reaction. J. Biol. Chem. **233:** 171–177.
4. SCHACHMAN, H. K., I. R. LEHMAN, M. J. BESSMAN, J. ADLER, E. S. SIMMS & A. KORNBERG. 1958. Physical chemical characterization of enzymatically synthesized deoxyribonucleic acid (DNA). Federation Proc. **17:** 304.
5. SCHACHMAN, H. K. 1957. Physical-chemical studies on deoxyribonucleic acid. J. Cellular Comp. Physiol. **49**(1): 71–81.

6. KUNITZ, M. 1950. Crystalline deoxyribonuclease. II. Digestion of thymus nucleic acid. The kinetics of the reaction. J. Gen. Physiol. **33:** 363–377.
7. WATSON, J. D. & F. H. C. CRICK. 1953. A structure for desoxyribose nucleic acid. Nature. **171:** 737–738.
8. WATSON, J. D. & F. H. C. CRICK. 1953. The structure of DNA. Cold Spring Harbor Symposia Quant. Biol. **18:** 123–131.
9. CHARGAFF, E. 1951. Structure and function of nucleic acids as cell constitutents. Federation Proc. **10:** 654–659.
10. WYATT, G. R. & S. S. COHEN. 1953. The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethylcytosine. Biochem. J. **55:** 774–782.
11. KORNBERG, A. 1959. Enzymatic synthesis of deoxyribonucleic acid. Harvey Lectures. Ser. **53:** 83–112.
12. SINSHEIMER, R. 1959. A single stranded deoxyribonucleic acid from bacteriophage ΦX174. J. Mol. Biol. **1:** 43–53.